

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
6 May 2004 (06.05.2004)

PCT

(10) International Publication Number  
**WO 2004/038038 A3**

(51) International Patent Classification<sup>7</sup>: **C12Q 1/68**,  
C12P 19/34, C07D 279/16

MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT,  
RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR,  
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(21) International Application Number:  
PCT/US2003/033429

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,  
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(22) International Filing Date: 22 October 2003 (22.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/420,717 23 October 2002 (23.10.2002) US  
60/439,978 14 January 2003 (14.01.2003) US

**Declaration under Rule 4.17:**

— as to the applicant's entitlement to claim the priority of the  
earlier application (Rule 4.17(iii)) for the following desig-  
nations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,  
BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC,  
EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,  
IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,  
LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ,  
OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,  
TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM,  
ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ,  
TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ,  
MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY,  
CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC,  
NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

(71) Applicants (*for all designated States except US*): UNI-  
VERSITY OF UTAH RESEARCH FOUNDATION  
[US/US]; 615 Arapleen Drive, Suite 10, Salt Lake City, UT  
84108 (US). IDAHO TECHNOLOGY, INC. [US/US];  
390 Wakura Way, Salt Lake City, UT 84108 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): WITTWER, Carl,  
T. [US/US]; 3479 South Crestwood Drive, Salt Lake City,  
UT 84109 (US). DUJOLS, Virginie, E. [FR/US]; 2119  
Royal Coventry Court, N° 31, Salt Lake City, UT 84121  
(US). REED, Gudrun [DE/US]; 1122 South McClelland,  
Salt Lake City, UT 84105 (US). ZHOU, Luming [CA/US];  
373 North H Street, Salt Lake City, UT 84103 (US).

**Published:**

— with international search report  
— before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

(74) Agent: POWLICK, Jill, T.; ICE MILLER, One American  
Square, Box 82001, Indianapolis, IN 46282-0002 (US).

(88) Date of publication of the international search report:  
22 July 2004

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE,  
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,  
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: AMPLICON MELTING ANALYSIS WITH SATURATION DYES

(57) Abstract: Methods are provided for nucleic acid analysis wherein a target nucleic acid that is at least partially double stranded is mixed with a dsDNA binding dye having a percent saturation of at least 50% to form a mixture. In one embodiment, the nucleic acid is amplified in the presence of the dsDNA binding dye, and in another embodiment a melting curve is generated for the target nucleic acid by measuring fluorescence from the dsDNA binding dye as the mixture is heated. Dyes for use in nucleic acid analysis and methods for making dyes are also provided.

WO 2004/038038 A3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/33429

**A. CLASSIFICATION OF SUBJECT MATTER**IPC(7) : C12Q 1/68; C12P 19/34; C07D 279/16  
US CL : 435/6, 91.2; 544/51

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 435/6, 91.2; 544/51

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	RIRIE, K. M. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Anal. Biochem., 1997, Vol. 245, pages 154-160. entire document, especially page 154.	1, 14, 16, 24-28, 31, 45-48 ----- 2-8, 13, 15, 17-23, 29-30, 32
Y	US 6,506,568 B2 (SHRIVER et al.) 14 January 2003, (14.01.2003), entire document, especially column 3, lines 20-67, column 4, lines 1-26	1-8, 13-28, 31-37, 39-48, 63-71, 74-75
Y	US 5,658,751 A (YUE et al) 19 August 1997 (19.8.1997), entire document	9-12, 49-69, 72-82
Y	US 6,437,141 B2 (RANDALL et al) 20 August 2002 (20.8.2002), entire document	9-12, 49-62, 72-82
X --- Y	US 6,174,670 B1 (WITTEWER et al.) 16 January 2001 (16.1.2001), entire document, especially, column 11, lines 10-38, column 22, lines 54-64,	1, 5-7, 13-14, 17-28, 30-33, 39-44 ----- 34-38, 45-47, 63-71

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.**\* Special categories of cited documents:**

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 April 2004 (16.04.2004)

Date of mailing of the international search report

25 MAY 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Suryaprabha Chunduru

Telephone No. 703-308-0196

**INTERNATIONAL SEARCH REPORT**

PCT/US03/33429

**Continuation of B. FIELDS SEARCHED Item 3:**  
Biosis, Embase, Caplus, Medline, Lifesci, EAST databases  
search terms: LCGreen, cyanine dyes, PCR, SYBR Green